The University of Chicago

MERISTEM IN OSMUNDA CINNAMOMEA

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

By
GEORGE LYNN CROSS

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CHICAGO, ILLINOIS

Reprinted from
THE BOTANICAL GAZETTE, Vol. CXI, No. 1, 1931

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MERISTEM IN OSMUNDA CINNAMOMEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 411

GEORGE L. CROSS

(WITH FIFTEEN FIGURES)

Introduction

The stele of the Osmundaceae has interested morphologists and anatomists for the past half century. DE BARY (9) first called attention to the fact that *Osmunda* in many respects exhibits a certain similarity to the type of cauline vascular system found in the majority of the seed plants. FAULL (10) described the adult anatomy of the group but did not attack the problem from the standpoint of the origin of tissues. JEFFREY (12) prepared a monograph in defense of his theory of the cortical origin of pith, using living and fossil forms of *Osmunda* to illustrate a series of advancement, beginning with *O. skidegatensis* (Jurassic) and ending with *O. claytoniana*. VAN TIEGHEM used practically the same series equally well in defense of his stelar theory. Despite the variation in interpretations of different investigators, however, it is generally conceded that the stele of *Osmunda* is very different from that of most other pteridophytes.

The present study was undertaken with the idea that a detailed investigation of the growing tips of the three prominent living species of *Osmunda* might throw light on the anatomical situation of the family as a whole. Although this paper is concerned almost entirely with *O. cinnamomea*, it is planned to make subsequent comparative studies of *O. claytoniana* and *O. regalis* before drawing conclusions relative to the phylogenetic anatomical situation of any of the species.

Material and methods

The material for the investigation was collected by the writer in the valley of the Calumet River, near Chicago.

Sections of the rhizomes were prepared for study by the paraffin method exclusively. To aid in cutting, the mass of sclerenchyma invariably found in the pith of *Osmunda cinnamomea* was dissected

out by making a longitudinal slit on one side of the stem through the cortex and stele when it was desired to cut sections any appreciable distance from the apex of the stem.

Much trouble was experienced in selecting appropriate killing and fixing reagents. Formalin-acetic-alcohol in any of the proportions recommended by CHAMBERLAIN[1] caused plasmolysis and rupture of the cells at the apices of root, stem, and leaf. Formalin-alcohol alone gave scarcely better results. Corrosive sublimate, hot or cold, occasionally gave good fixing, but as a rule failed entirely. Chromo-acetic acid penetrated very poorly and in many instances caused maceration of the material. Perhaps the most satisfactory killing and fixing reagent employed was 10 per cent neutral formalin, although some destruction of the large apical cells occurred by this method.

General anatomy

The rhizome is a massive, semisubterranean, slow growing structure, having heavy leaf bases and an outer cortex impregnated with sclerenchyma. The stem branches, probably dichotomously. The leaves arise at the apex, and in acropetal succession form a close spiral, the phyllotaxy of which has been worked out by FAULL (11). The leaf traces are concentric and endarch. The stem, to use JEFFREY'S nomenclature, is a phyllosiphonic mesarch siphonostele, varying from the amphiphloic condition (*O. skidegatensis* and sometimes *O. cinnamomea*) to the ectophloic (*O. regalis* and *O. claytoniana*). Maturation at the tip of the rhizome proceeds slowly, and in consequence all tissues mature at a relatively great distance from the apex, although the xylem and protophloem are differentiated early. The roots arise opposite the wings of metaxylem, two for each leaf trace in *O. regalis* and *O. claytoniana*, and but one in *O. cinnamomea*.

STEM TIP

An apical cell or a group of horizontally seriated initials is always present. The first major work on the stem apices of the Osmundaceae was done by BOWER (2), who investigated *O. regalis* in detail. He describes the growing tip and reports finding a four-sided initial cell in a few apices. He states that there is present in most forms a

[1] Methods in plant histology. Chicago. 1915.

conical apical cell which is rather deep in proportion to its height, but he concludes that the apical meristem on the whole is of a "less regular and definite type than in the leptosporangiate ferns."

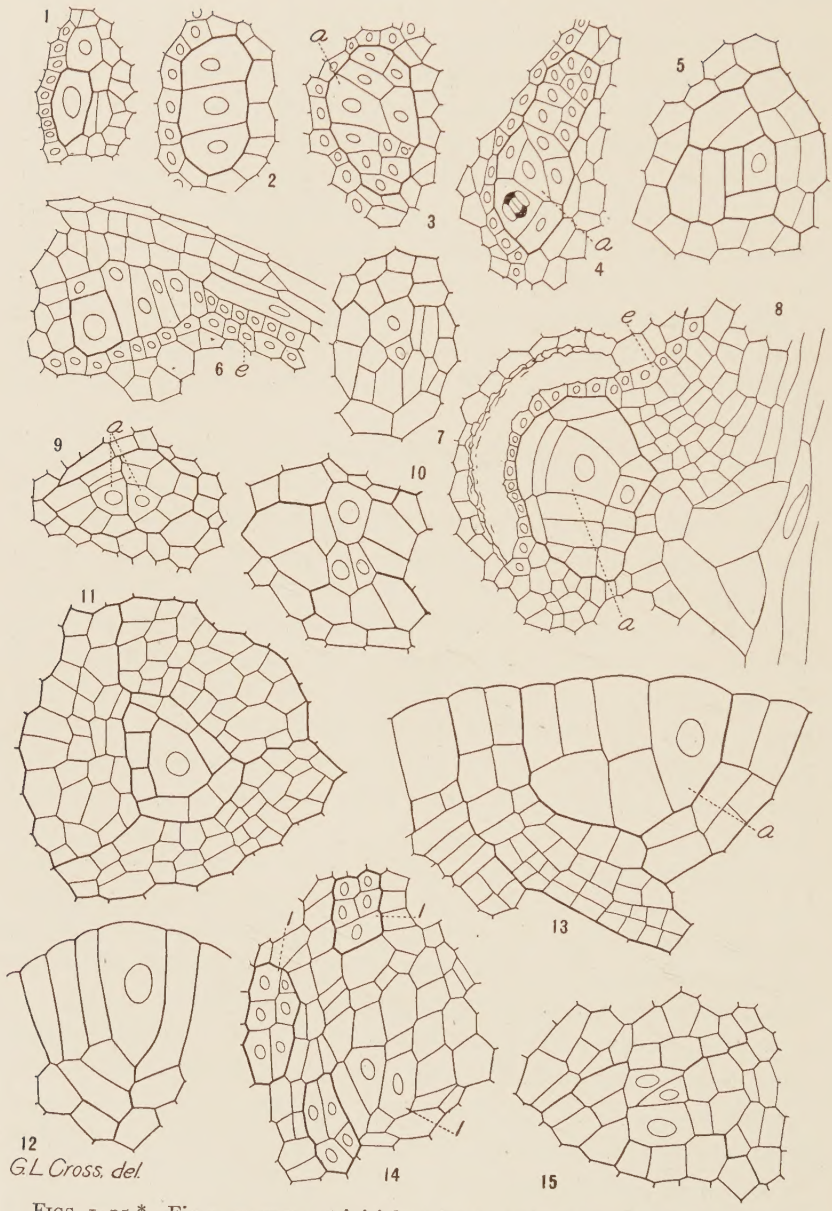
My investigations were concerned almost exclusively with *O. cinnamomea*, although a sufficient number of preparations of *O. regalis* were made to verify the work of BOWER. In general topography, the apex of *O. cinnamomea* is much the same as the apex of *O. regalis*. The cells are relatively large, thin walled, and watery.

The apices of a great number of rhizomes were sectioned. Plants of all ages and sizes were selected, from the sporeling stage up to extremely old rhizomes. Both longitudinal and transverse sections were prepared.

In the sporeling, as noted by CAMPBELL (7), a triangular pyramidal apical cell is prevailingly present. This cell persists during the youth of the rhizome, and frequently may be found in middle aged plants. The relative size and shape of the cell are shown in fig. 11. Obviously the segmentation does not proceed with the regularity characteristic of the typical leptosporangiate fern. The segments are not cut off in spiral succession as a rule, although this mode of growth sometimes occurs intermittently for a small number of segments (4-6). The identity of the segments is soon lost, due to the relatively great length of time elapsing between successive segmentations, and to the numerous divisions occurring in the segments themselves.

Before the young rhizome forks, the apical cell apparently divides into two equal portions, in each of which is organized a new apical cell that functions as the growing point of the divergence. Fig. 9 was drawn from the growing tip of a young rhizome, which was about to fork in the dichotomous manner described.

As the rhizome ages, segmentation at the apex becomes progressively less regular, and differential growth, or twisting of the stem tip due to growth of the leaves, may pull the apical cell into such position that it appears to be four-sided (fig. 14). As the segmentation becomes more and more irregular, the identity of the original triangular pyramidal apical cell becomes lost, and it is replaced by a group of initials which function as a meristem (fig. 10). The old rhizomes may branch, but infrequently. FAULL records *Osmunda* as



FIGS. 1–15.*—Fig. 1, young root initial arising in pericycle just within endodermis (at left); cross-section of rhizome. Fig. 2, young root in 3-celled stage with endodermis

* All figures drawn at level of table with a Spencer camera lucida, under a 4 mm. Leitz apochromatic objective, with periplan ocular 8×; reduced to one-fourth original size.

branching once. I have observed several cases in which a single rhizome branched three times. After branching, the older portions of the rhizome decay with the advent of time and two individuals result. Such a method of reproduction is characteristic of practically all plants with an underground branching stem. Fig. 13 is a representative longitudinal view of the apex of a rhizome which is on the verge of losing its growth by means of an apical cell, in favor of growth by meristem of two or three cells. To the left of this figure is shown the origin of desmogen tissue and a possible leaf initial. The sequence of segments may be traced here to better advantage than in any other rhizome studied. It can be seen that the segmentation has been regular, and almost of the leptosporangiate type until the last three or four segments, when the apical cell was apparently thrust to one side by the overgrowth of one of the lateral derivatives. This lateral derivative then, instead of undergoing the usual periclinal division, divided in a plane perpendicular to the surface of the apex, resulting in the formation of two cells approximately equal in rank to the apical cell. Extensive studies of like situations would indicate that this is a preliminary step in the replacement of the apical cell with a meristematic group. The other

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to left; cross-section of rhizome. Fig. 3, longitudinal section of young root with definite apical cell (*a*); segments which will contribute to formation of root cap not yet formed. Fig. 4, longitudinal section of young root with definite apical cell which has cut off initial cell of root cap to left; endodermis at left. Fig. 5, cross-section of young root passing through cortex of rhizome; sequence of segments obviously irregular. Fig. 6, longitudinal section of rhizome showing enlarged root initial being formed in pericycle just within endodermis (*e*). Fig. 7, cross-section of young root entering soil; individuality of original apical cell is being lost, and a 2- or 3-celled meristem apparently taking its place. Fig. 8, longitudinal section of rhizome and young root showing truncate appearance of 5-sided apical cell, and portion of leaf trace with which vascular system of root will ultimately become continuous. Fig. 9, cross-section of apex of young rhizome just before forking, showing apical cells of the two branches. Fig. 10, cross-section of apex of old rhizome with apparently 3-celled meristem. Fig. 11, cross-section of apex of very young rhizome showing definite tetrahedral apical cell which has undergone rather definite segmentation; leaf initials not distinguishable. Fig. 12, longitudinal section of apex of young rhizome with definite tetrahedral apical cell. Fig. 13, longitudinal section of old rhizome; growth slow and individuality of original apical cell practically lost; elongated cells which later become desmogen tissue at left. Fig. 14, cross-section of apex of old rhizome; irregular triangular apical cell represented with three young leaves (*l*) and possible initial of fourth. Fig. 15, cross-section through apex of old rhizome showing one of many irregular types of meristems found in this species.

possibility remains, however, that this is merely an expression of one of the many irregularities that may be expected in the apical meristem of any of the Osmundaceae, even if a functioning apical cell be present.

Whatever the type of segmentation, lateral derivatives of the apical or master cells divide, at first periclinally, and then in three planes in such a manner as to form two or four large outer cells and a number of smaller inner cells for each segment (fig. 13). The latter elements form a solid dome-shaped structure, the peripheral members of which give rise to desmogen tissue, unbroken by leaf gaps. The larger outer cells mentioned in connection with the lateral derivatives of the apical cell undergo a few anticlinal divisions, and ultimately certain of their progeny give rise to leaf initials, while the remainder contribute to the undifferentiated epidermis of the leaf.

Root origin and development

An effort was made to determine the histological relationships of the tissue in which the root initial appears, but, due to the slow differentiation and maturation of the stem, combined with the early origin of leaf traces, decisive information was not obtained. A number of factors indicate that the young root arises in a tissue which is on the border line between the stele and cortex, and which later becomes differentiated into pericycle (figs. 1, 6). Studies in this connection indicate that this tissue may not be traced to any definite cell derived from segments cut off by the apical cell or meristematic group.

The initial cell of the root may be observed very near the tip of the rhizome. It is conspicuous on account of its large size and by its nucleus, which is usually nearly twice as large as the nuclei of the surrounding cells (fig. 1).

As in the case of the stem, development of the root is characterized by extreme irregularity. Two, or even three initial cells may be involved in the origin of the root, one of which may give rise to an apical cell, or the group may form an irregular meristem. After examination of a great number of rhizomes, the conclusion was reached that the mechanics involved in probably more than half of the cases are in accordance with the following description: Shortly

after the root initial is differentiated, it undergoes two divisions in a plane parallel, or nearly parallel to the longitudinal axis of the rhizome and perpendicular to its surface. Due to the growth of the leaf trace, with which the vascular system of the root will ultimately become continuous, the resulting cells may be slightly stretched in a plane oblique to the axis of the rhizome, a fact which makes it extremely difficult to obtain median sections, regardless of how the rhizome be oriented in cutting. The three cells obtained by the two divisions just described often undergo a period of enlargement before further divisions occur (fig. 2). The surrounding tissue continues to divide, thus keeping pace with the enlargement of the root initial and the growth of the leaf trace with which the vascular system of developing root becomes continuous.

In *Osmunda cinnamomea* the prevailing situation is one root for each leaf, but a few instances were observed in which two root initials had been formed for a single leaf; one of them, however, proceeded only to the formation of a few cells before its growth was inhibited.

After the three-celled stage the young root develops rapidly. An oblique wall is laid down in the central cell, resulting in the formation of a wedge-shaped element which functions as the apical cell of the young root. The lateral members of the original three also divide into a number of more or less rectangular cells (fig. 3). Sometimes one of these lateral cells may divide in a manner suggestive of an apical cell (fig. 4), but its activities are soon altered and subordinated if the original apical cell is to retain its identity. If these lateral cells divide and keep pace with the apical cell, a multicellular meristem results.

The apical cell of the young root just described has five cutting faces. It may be triangular in profile, or truncate at the base (fig. 8). The four posterior faces cut off segments which form the body of the root, but contribute also to the root cap, while the fifth anterior face contributes to the formation of the root cap exclusively. Fig. 8 was drawn from a longitudinal section of a young root. The apex of the root is capped by a layer of cells which are probably endodermal, but have not been derived from the apical cell of the root. Such a situation is frequently found in angiosperms, where the lateral roots

are distinctly pericyclic in origin. The layer of cells, which may be the undifferentiated endodermis, is easily distinguished at the time the root initial is set off from the rest of the tissue (figs. 1, 6). Its histogenetic relationship is not clear, as already indicated, due to the frequent interruptions of the continuity of the stele by leaf gaps. It should be noted, however, that at the time of the origin of the root initial the pericycle consists of but one layer of cells (fig. 6), and is distinct from the endodermis. Tracing such sections upward toward the apex of the rhizome has resulted in strong but not positive evidence for the independent origin of the pericycle and endodermis.

A few cases were observed in which a triangular pyramidal apical cell might have been present. The same condition has been reported for the older roots of *Osmunda regalis* by BOWER (3). In none of the cases, however, did the triangular cell function with the diagrammatic regularity so characteristic of the leptosporangiate ferns. Such apices are regarded as possessing a meristematic growing tip (fig. 7) rather than a triangular apical cell. The majority of the young roots had a five-faced apical cell, as previously described.

Segmentation may be counter-clockwise for a time (three or four segments), but as the root enlarges and starts to pass through the cortex of the rhizome, the walls seldom follow in the typical spiral succession. Fig. 5 represents a cross-section of such a root. The irregularity of placement of walls may readily be observed, and deemed doubly significant, inasmuch as, of the fifteen or twenty cases examined, this root was selected as having the most regular segmentation. Irregularity increases with age, and in old roots the single apical cell gives rise, in many instances, to a pair, three, or even a group of initials, horizontally seriated and of approximately the same rank. This irregularity, which results in the loss of the apical cell, is not confined to the root, but is also characteristic of the stem, as was shown previously. Such evidence suggests that *Osmunda* is losing its growth by means of an apical cell, in favor of a type of growth exemplified by some of the higher plants, and more characteristic of massive stems. The evidence is distinctly in support of the views set forth by BOWER (3).

There has been no satisfactory work to date on the histogenesis

of the roots of the Osmundaceae. BOWER describes the meristem of the root of *Osmunda* as being subject to variation, and states:

> since it is hardly possible to determine with certainty from longitudinal sections what is the actual number and form of the initial cells, a full and exact interpretation of the structures in longitudinal sections cannot be expected.

Most of the sections examined by the writer were in support of this conclusion.

Discussion

Nearly a half century ago BOWER (3) made a comparative study of the meristems of a group of families which are included with the Filicales, namely, Hymenophyllaceae, Polypodiaceae, Cyatheaceae, Schizaeaceae, Osmundaceae, and Marattiaceae. His chief contribution was to demonstrate a parallelism in character of all the meristems in these divisions, and to show that in the first named groups the meristems are simple in construction, consisting of an apical cell which exhibits the definite regularity of segmentation also characteristic of the Equisetales. Passing upward through the series, BOWER found that regularity of segmentation is no longer characteristic of the growing points, and that the identity of the single initial is lost as the sequence of segments becomes less definite. The list of families just given is taken from his paper as representing a natural series which grades from the relatively simple to the more complex families of the Filicales.

BOWER regards the Osmundaceae as bridging the gap between the leptosporangiate and eusporangiate ferns. He bases his evidence on a study of the meristems of adult plants, and on certain structural and developmental characteristics of the sporangia. He found that the true leptosporangiate ferns have meristems characterized by an apical cell, that is, by a single initial, and that the sporangium initial is also a single cell. The Marattiaceae have meristems with many initials, in some instances not unlike the growing tips of some modern angiosperms and gymnosperms. The sporangium arises from a group of cells rather than from a single initial. With respect to these characters the Osmundaceae occupy an intermediate position, with their irregular meristems and sporangium initials.

It was thought that perhaps the evidence concerning the meristems of the Osmundaceae might be better evaluated if a study of

the origin of the meristems were made, inasmuch as evidence of the ancestral condition may be found sometimes in the embryonic state. It has been stated already that the stem of the sporeling grows by means of an apical cell which segments in a more or less regular manner. This suggests the leptosporangiate type. As the rhizome ages, irregularities appear and a multicellular meristem frequently results, which resembles the stem tip of certain of the Marattiaceae, more definitely *Marattia* itself. A similar situation has been reported for *M. alata* by CHARLES (8), who describes the stems of young sporelings of this species as having a single irregular initial with an early transition to the complex meristem. The root initial prevailingly gives rise to a five-sided apical cell with five cutting faces, but occasionally a tetrahedral apical cell with four cutting faces is developed. This condition is the one found among leptosporangiate forms. As the root develops, the identity of the apical cell is lost and a type of growth appears which suggests the condition found in the Marattiaceae. Thus the evidence derived corroborates the conclusions of BOWER, and in addition suggests that the Osmundaceae are a transitional group; that they are at the present time losing leptosporangiate characteristics and assuming a type exemplified by the eusporangiate fern.

Although the evidence indicates that *Osmunda* is changing from one type of meristem to another, that is, from the leptosporangiate to the eusporangiate type, it throws no light upon the relative antiquity of the two groups. At first thought such evidence might appear to favor the earlier conception of BOWER, that the eusporangiates are the more recent of the groups, but it seems as logical to assume that the Osmundaceae may be passing from a relatively recent leptosporangiate type to the goal attained by modern angiosperms, rather than forward to the eusporangiate level.

The sequence of families listed by BOWER (3) was arranged on a basis of comparison of living representatives, and paleobotanical evidence was neglected, as he suggests in some of his later works (5). As early as 1891, CAMPBELL (6) argued in favor of the eusporangiate ferns as being the more primitive of the two groups. Since that time numerous papers have appeared, many of which have taken paleontological evidence into consideration. To review this literature would be beyond the scope of this paper, and it will suffice to say

that the evidence is now almost preponderantly in favor of the relative primitiveness of the eusporangiate forms. That the Osmundaceae are not contradictory in this respect has been indicated here.

If we may interpret embryonic structures as representing a condition of the adult extant in the past, it seems obvious that *Osmunda* is changing, in that it is apparently becoming more complex. From the description of Campbell (7), *O. claytoniana* seems the least changed of the three species with respect to meristems, inasmuch as it retains the leptosporangiate characters to a greater degree, especially in the root. In this light, excluding anatomical evidence for the moment, it appears feasible to place *O. claytoniana* as the most primitive of the three species, instead of as the most advanced, as suggested by Jeffrey (12). *O. regalis* and *O. cinnamomea* appear to be at about the same phylogenetic level from the standpoint of meristems.

Thus two interpretations may be made on the evidence presented by *Osmunda*. If the Filicales are considered to the exclusion of the spermatophytes, *Osmunda* shows evidence of retrogression and of attaining the primitive eusporangiate type. If the spermatophytes are taken into consideration, *Osmunda* may be thought of as progressing and of approaching the goal exemplified by a number of the angiosperms, a view which is at present more acceptable.

Summary

1. The rhizome of *Osmunda cinnamomea*, when in the sporeling stage, grows by means of a tetrahedral apical cell. As the plant reaches maturity the segmentation of the apical cell becomes progressively less regular, in the majority of cases, until a condition is reached where the identity of the original apical cell is lost, and growth takes place by the activitity of two or more initials.

2. Cells which are destined to become desmogen tissue may be recognized in the second segment from the apical cell.

3. Due to the slow growth of the rhizome and the frequency of leaf gaps, it is extremely difficult if not impossible to refer any portion of the mature rhizome to any definite portion of the segments from the apical cell or group of initials.

4. The pericycle and endodermis apparently have independent origins, although decisive evidence was lacking. The roots arise in

tissue which later gives rise to the pericycle. The undifferentiated endodermis acts as a sort of protective layer to the young root.

5. The number of cells of most young roots is increased for a time by means of an apical cell with five cutting faces. As in the case of the stem, progressive irregularity in segmentation of this cell often results in the formation of two or more initial cells which replace the apical cell.

6. The evidence obtained is in support of the conclusions of BOWER (**3, 5**) that the Osmundaceae occupy an intermediate position between the leptosporangiate and the eusporangiate ferns, but it throws no light upon the relative antiquity of the two groups.

It is a pleasure to acknowledge the aid of Professor W. J. G. LAND, who suggested the problem, helped with the collection of material, and made suggestions concerning the interpretation of the preparations. I am deeply grateful to Professor E. J. KRAUS for his kind criticism of the manuscript and subject matter.

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[*Accepted for publication January 10, 1930*]

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